"STEREO-CHROMATOGRAPHY" A NEW METHOD FOR CHEMICAL IDENTIFICATION AND PREPARATIVE ISOLATION

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Since the initial reports on chromatographic techniques[1], and especially following the investigations of Consden, Gordon, and Martin[2], and Martin and Synge[3], numerous modifications of these procedures have made possible remarkable advances in biochemical research. By evaluation of migration characteristics of unidentifiable with identifiable solutes separated by partition chromatography, identification of unknown solutes is facilitated. Similarly, by utilizing preparative "filter paper pile" techniques, such as the chromatopile[4,5], the chromatopack[6], or the chromatoblock[7], constituents of concentrated mixtures can be chemically resolved to yield semimicro-quantities of pure materials. The present communication concerns the feasibility of utilizing three-dimensional, compressed, paper pulp blocks for preparative chemical isolation and identification. This technique affords three distinct advantages: (1) It makes possible the analysis of an additional parameter, the "spreading factor, R_S", which can be used to chemically differentiate constituents of the solute mixture along with conventional R_F values; (2) it affords a support system capable of handling and separating exceptionally concentrated loads of solute mixtures for subsequent semimicro-determinations; and (3) it provides a closed system which should inhibit oxidations or photochemical alterations of solute

zones which sometimes occur in conventional, paper chromatography. One disadvantage of the technique is that it requires a solid, compressed fiber block, which, although inexpensive to fabricate, is not yet commercially available. In addition, the procedure necessitates the use of an electric band or jigsaw for serial sectioning of the chromatographic block.

Preliminary chromatographic experiments were undertaken with compressed paper pulp* blocks. The blocks were fabricated by compressing raw, 3-4 per cent, paper pulp compressed at 3,000 pounds per square inch in a metal sieve box by means of a Dake Hydraulic Press. After compression, which removed most of the water, the blocks were oven dried for 36 h at 70° C. It was found in previous compression experiments that homogenization of the crude paper pulp in a Waring blendor prior to compression increased the homogeneity of chromatographic blocks.

The feasibility of stereo-chromatography was shown by the three-dimensional separation of indicator dyes in simple ascending chromatography. According to the procedure of Porter[8], indicator dyes were used as solutes in the organic phase of an equilibrated two-phase solvent mixture obtained by mixing 40 parts of 1-butanol, 10 parts of absolute ethyl alcohol, and 50 parts of water. This mixture was separated in a separatory funnel and the hyperphase was used.

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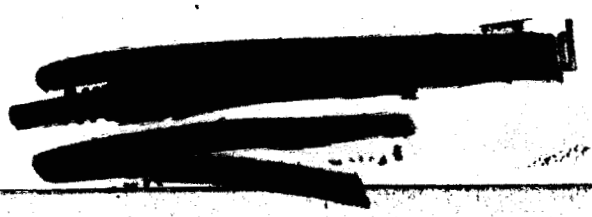
*Paper pulp was purchased from the Container Corporation of America, Santa Clara, California.



In one early exploratory experiment, 1.5 ml. of an aqueous solute mixture containing 1.5 mg bromcresol green, 1.2 mg bromothymol blue, and 1.0 mg eosin blue was injected into the center of the base of a 25 cm × 20 cm × 7.5 cm compressed pulp block. The block was placed on the bottom of a large, closed jar containing 1/2 inch of solvent mixture, and was exposed for 3 hours, or until the solvent front almost reached the top of the block.

At the termination of the experiment, the block was removed from the solvent and serially sectioned by an electric band saw into 0.5 cm sections. Since the block cannot be readily dried internally, it is necessary to section wet blocks in order to prevent solute zone migration which might occur during prolonged drying.

After the stereo-chromatogram was sectioned, the vertical and lateral migrations of the solute zones were determined on each section by means of a metric calibrated, transparent, plastic grid. The vertical migration (R_F) values were determined in the conventional manner. The lateral migration or spreading factor of the solute zones was determined by calculating the ratio of the lateral spread of the solute from the central axis of the block to the vertical migration of the solvent front. This parameter was termed the "R_S value".

It was observed in most stereo-chromatographic blocks that zone resolution was adequate to isolate and identify the specific indicator dyes utilized. Of greater significance, however, is the fact that

differences in lateral spreading (R_S values) of the indicator dyes were observed as shown in Table 1 which includes data from four recent experiments.

Table 1. VERTICAL AND LATERAL MIGRATION IN STEREO-CHROMATOGRAPHIC BLOCKS

Solute	R_F	R_S
Methyl orange	0.32	0.08
Methyl red	0.68	0.24
Bromothymol blue	0.79	0.35
Methylene blue	0.88	0.04
Eosin blue	0.01	0.04

The present preliminary experiments indicate that stereo-chromatography with compressed paper pulp blocks offers a suitable technique for preparative chromatography where large amounts of solutes are to be resolved. This procedure should therefore prove useful in biological, and possibly industrial purifications.

Although additional experiments are necessary to critically evaluate the relation of R_S to R_F values, it is most noteworthy that the spreading factor, R_S, offers an additional parameter to differentiate chemical constituents. Such a parameter should be of practical importance in differentiating compounds which have similar partition coefficients, and thus produce similar R_F values (e.g., leucine and iso-leucine, valine and nor-valine), but which differ in carbon chain configuration. In conventional partition chromatography, Fisher and his associates[8] experimentally demonstrated that a linear relation

holds between the area of the spot of a test substance and the logarithm of its original concentration. Brimley[9] has theoretically discussed this relationship by applying equations analogous to those developed in the theory of heat flow, and by assuming that the spot moves along the paper chromatogram (conventional chromatography) by simple diffusion. However, it is obvious in the present preliminary stereo-chromatograms that such a simple relationship does not apply, analogously, to the volume of solute zones and their original concentration. It appears that the greater differences in R_S than in R_F migrations for a given solute pair (bromothymol blue and methylene blue) may indicate that lateral vectorial forces which influence solute zone movement (e.g., solubility and/or ordinary diffusion, eddy diffusion, and local non-equilibrium) become more apparent in horizontal spreading than in vertical migration. In the latter case the capillarity and opposing gravitational forces are predominant. In other instances lateral, diffusive forces appear less predominent (methylene blue and eosin blue).

As in all chromatographic techniques, homogeneity of the supporting medium is requisite for regular movement of solvent and solutes. The blocks in the present experiments were fairly homogeneous, but less so than ordinary commercial filter paper. Fiber size can be expected to influence solvent flow rate in stereo-chromatography as it does in paper chromatography. Similarly, the initial flow rate (first 20 min) was found to be faster than in later periods in stereo-chromatography, as it is in conventional chromatography.

The authors explored the use of a new chromatographic parameter, lateral migration, through the use of a solid compressed fiber block. The suggested system has the advantage of being able to support concentrated loads of solutes and of providing a closed system which should inhibit oxidations or photochemical alterations of solutes.

Although only dyes were used in this study we have been able to make somewhat the same observations with other solutes.

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